Mutation analysis was accomplished as follows. Initial searching for the HH mu tion in cDNA24 was accomplished through RT-PCR (reverse transcription-polymerase chain reaction, Dracopoli, N. et al. eds. Current Protocols in Human Genetics (J. Wiley & Son New York (1994)) method. First, from the genotype analysis, homozygous HH patients ith the ancestral haplotype were identified (see previous sections). First strand cDNAs were synthesized through use of SUPERSCRIPT™ reverse transcriptase (Invitrogen Life Technologies, Carlsbad, CA) using polyA+ RNA from transformed lymphoblastoid cell nes from two homozygous ancestral patients (HC9 and HCI4) and those from two unaffected individuals (NY8 and CEPH 11840) as templates.

A1

Please delete the paragraph beginning at page 26, line 33 of the Specification and replace it with the following paragraph:

Amplified DNA products (PCR-products) were purified usingGELASE™ (Epice re, Madison, WI), and DNA sequences of these PCR-fragments were determined by the did xy chain termination method using fluorescently labeled didcoxy nucleotides on an ABI 37 DNA sequencer (Applied Biosystems, Foster City, CA).

A2

Please delete the paragraph beginning at page 32, line 3 of the Specification and replace it with the following paragraph:

cDNA 24 tissue expression was determined by probing polyA+ RNA Northern b ts (Clontech, Palo Alto, CA). One major transcript of approximately 4.4 Kb was observed all of the 16 tissues tested including small intestine and liver.

A3

Please delete the paragraph beginning at page 32, line 6 of the Specification and replace it with the following paragraph:

The genomic region corresponding to cDNA 24 was cloned and sequenced . CDNA 24 is comprised of apparently seven exons, covering approximately 11 Kb of sequence. The putative seventh exon is completely non-coding and contains one poly (A)+ addition signal. In the region of the predicted start site of transcription, there are no consensus CAAT or TATA boxes, nor are there any start like βGAP-like sequences recently suggested by Rothenberg and Voland, *supra* (1996). One CpG island was found to overlap the first exon and extend into the first intron. Within this island are the consensus cis-acting binding sites for the transcription factors Sp1 (2 sites) and AP1 (1 site) (MACVECTOR™ software, Oxford Molecular, San Diego, CA). The lack of any recognizable TATA boxes and the presence of Sp1 and AP2 binding sites is consistent with the low level of transcription associated with the gene.

A4

Please delete the paragraph beginning at page 49, line 30 of the Specification and replace it with the following paragraph:

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells (Maniatis et al. *supra.* (1982); Sambrook et al. *supra.* (1989); Meth. Enzymology *supra.* (1979, 1983, 1987); U.S. Patent No. 4,399,216; Meth Enzymology *supra* (1986); Gelvin et al. *supra.* (1990)). Such techniques include, without limitation, calcium treatment employing calcium chloride for prokaryotes or other cells which contain substantial cell wall barriers; infection with *Agrobacterium tumefaciens* for certain plant cells; calcium phosphate precipitation, DEAE, lipid transfection systems (such as LIPOFECTIN™ and LIPOFECTAMINE™, Invitrogen, Carlsbad CA), and electroporation methods for

A5

A5

mammalian cells without cell walls, and, microprojectile bombardment for many cells including, plant cells. In addition, DNA may bc delivered by viral delivery systems such retroviruses or the herpes family, adenoviruses, baculoviruses, or semliki forest virus, as appropriate for the species of cell line chosen.

Please delete the paragraph beginning at page 58, line 1 of the Specification and replace it with the following paragraph:

A6

Protein replacement therapy requires that RH protein be administered in an appropriate formulation. The HR protein can be formulated in conventional ways standard to the art for the administration of protein substances. Delivery may require packaging in lipid-containing vesicles (such as LIPOFECTIN™ or other cationic or anionic lipid or certain surfactant proteins) that facilitate incorporation into the cell membrane. The RH protein formulations can be delivered to affected tissues by different methods depending on the affected tissue. For example, iron absorption is initiated in the GI tract. Therefore, delivery by catheter or other means to bypass the stomach would be desirable. In other tissues, IV delivery will be the most direct approach.

Please delete the paragraph beginning at page 65, line 5 of the Specification and replace it with the following paragraph:

A7

In amplification, a solution containing the DNA sample (obtained either directly or through reverse transcription of RNA) is mixed with an aliquot of each of dATP, dCTP, dGTP and dTTP (i.e., Pharmacia LKB Biotechnology, Piscataway, NJ), an aliquot of each of the DNA specific PCR primers, an aliquot of *Taq* polymerase (i.e., Promega, Madison, WI), and an aliquot of PCR buffer, including $MgCl_2$ (i.e., Promega) to a final volume. Followed

by pre-denaturation (i.e., at 95°C for 7 minutes), PCR is carried out in a DNA thermal cycler (i.e., Perkin-Elmer Cetus, Shelton, CT) with repetitive cycles of annealing, extension, and denaturation. As will be appreciated, such steps can be modified to optimize the PCR amplification for any particular reaction, however, exemplary conditions utilized include denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 4 minutes, respectively, for 30 cycles. Further details of the PCR technique can be found in Erlich, "PCR Technology," Stockton Press (1989) and U.S. Patent No. 4,683,202, the disclosure of which is incorporated herein by reference.

A7

Please delete the paragraph beginning at page 73, line 6 of the Specification and replace it with the following paragraph:

A8

The PCR is performed in standard PCR-reaction buffer (e.g., 1X GENEAMP® reaction buffer from Applied Biosystems, Foster City, CA, with 1.5 mM Mg^{++}) for 35-30 cycles using an annealing temperature of 60°C.

IN THE CLAIMS

Please cancel Claim 1, without prejudice against Applicants' right to prosecute claims drawn to the canceled subject matter in one or more timely-filed continuation, divisional and/or continuation-in-part applications.

Please add new Claim 92-137:

A9

92) (New) A plasmid comprising a nucleic acid sequence selected from the group consisting of:

(a) the nucleic acid sequence of SEQ ID NO:1,

(b) the nucleic acid sequence of SEQ ID NO:3,